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Study the relationship between NFKB-p65 and some immunological biomarker in patients infected with Toxoplasma Gondii parasite

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***Abstract*---**The study was conducted on 350 aborted women and 30 healthy women who have visited Public Health Laboratory, in Babylon Governorate from August2021 to February 2022. The current study revealed that serum concentration of NF-$_{KB}$,IL-18 (pg/ml), IL-1β (pg/ml) and TNF-α (pg/ml) in patients infected with *Toxoplasma gondii* were significant increase (P< 0.001) (18. 812 ± 0.185 ng/ml), (474. 12338± 0.906 pg/ml), (15. 41450± 0.218 pg/ml), (25. 4145065284± 0.218232 pg/ml) in compared to the control group (8.515 ± 0.515 ng/ml), (97.107565 ± 0. 700 pg/ml), (4.129558 ± 0. 190 pg/ml), (6.45839 ± 0. 220 pg/ml). The current study has concluded that the infection with *T. gondii* affects the body's immunity by NF-$_{KB}$, IL-18, IL-1β and TNF-α. In addition, study was designed to reveal the correlated between NF-$_{KB}$ with IL-18, IL-1β and TNF-α in patients infected with *T. gondii.* The results of the current study showed the serum concentration of NF-$_{KB}$ was correlated positively and significantly with serum concentration of IL-18, IL-1β and TNF-α.

Keywords*---Toxoplasma gondii*, IL-1β, NF-$_{KB}$, Kufa, abortion.

Introduction

T. gondii is the causative agent of toxoplasmosis that is a zoonosis of significant medical and veterinary importance and is transmitted by several pathways. Marked advances regarding the control of several infectious diseases caused by parasitic protozoa have taken place in the last decades, especially those that spend part of their life-cycle inside host cells. Like toxoplasmosis, are widely distributed throughout the world (Robert-Gangneux & Dardé.2012). Indeed, *T. gondii,* a member of the phylum Apicomplexa, developed the ability to infect

almost any cell type of mammals and birds (Hill & Dubey, 2002). In some geographical areas (e.g. Brazil), up to 60% of the population is seropositive for *T. gondii* antigens (Dubey *et al.*, 2012). The environmental conditions and dietary habits can impact infection rates. For example, the ingestion of raw or undercooked meat is associated with *T. gondii* transmission, and pig and sheep meat are more prone to contain tissue cysts than cattle (Tenter *et al.*, 2000; Stelzer *et al.*, 2019). NF-$_{KB}$ is a transcription factor; a family of five proteins can dimerize to form NF-$_{KB}$ complexes: NF-$_{KB}$ 1 (p105), NF-$_{KB}$ 2 (p100), RelA (p65), RelB and c-Rel (Lu, & Stark. 2015). NF-$_{KB}$ 1 and NF-$_{KB}$ 2 undergo processing into mature forms, p50 and p52, respectively. All the NF-$_{KB}$ proteins share the Rel homology domain (RHD) which allows them to form homo- or heterodimers (Lu, & Stark. 2015) and bind DNA (Giuliani *et al.*, 2018). TNF-α is secreted by different cell kinds including immune cells like (B cells and T cells, natural killer cells, basophils, dendritic cells, eosinophil, neutrophil and mast cells), non-immune cells (astrocytes, granuloma cells, fibroblasts, glial cells, and keratinocytes) (Bradley *et al.*, 2008). The innate immune response to *T. gondii* has the capability to sense the pathogen and secrete the IL-12, which motivates natural killer (NK) cells and T cells to secrete the interferon-gamma (IFN-γ) (Hunter *et al.*, 1994& Zaba *et al.*, 2007). IL-1β is produced by hematopoietic cells such as blood monocytes, tissue macrophages, skin dendritic cells, and brain microglia in response to TLR, activated complement components, other cytokines (such as TNF-a), and IL-1 itself (Dinarello, 2011). IL-18 can contribute to some of the manifestations of inflammasome-mediated caspase-1 activation in autoinflammatory diseases (Towne *et al.*, 2011).

The subjects

The study was conducted on 350 suspected patients with *T. gondii* parasites and 30 healthy people as control groups. The institutional ethics committee approved collecting samples of the faculty of science at the University of Kufa, and all participants signed informed consent forms. Toxo specific IgM and IgG methods examine all suspected samples. These samples were collected from suspected patients who attended the AL-Zahra maternity and paediatrics hospital in AL-Najaf province from August to January 2022.

Blood Specimens collection

From August to January 2022, there were only 60 positive samples out of 350 suspected patients and 30 healthy who attended the AL-Zahra maternity and paediatrics hospital clinics in AL-Najaf province. The blood samples were collected from patients by vein puncture into specimen tubes and remains for 30 minutes at room temperature. After that, the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany**)** to separate the serum and collected in other sterile tubes; each sample of serum was divided into five parts; each of them was kept in deep freeze at -20C° till used for the determination of NF-$_{KB}$, IL-18, IL-1β and TNF-α

The Kits

The biomarkers in the current study were estimated by Eliza Kits such as Human NF-$_{KB}$ ELISA/ Elabscience / (Catalog No: E-EL-H1388), Human Interleukin-18 (IL-18) ELISA/ Elabscience / (Catalog No: E-EL-H0253), Human Interleukin-1β (IL-1β) ELISA/ Elabscience / (Catalog No: E-EL-H0149), Human Interleukin-1β (IL-1β) ELISA/ Elabscience / (Catalog No: E-EL-H0109).

Statistical analysis

Data were analyzed using the software packages Graph pad prism for Windows (5.04, Graph pad software Inc. USA); data are presented as the mean ± standard error (SE). The comparison between the patients and control groups were analyzed by student t-test.

Results

The current study revealed that serum concentration of NF-$_{KB}$,IL-18 (pg/ml), IL-1β (pg/ml) and TNF-α (pg/ml) in patients infected with *Toxoplasma gondii* were significant increase (P< 0.001) (18. 812 ± 0.185 ng/ml), (474. 12338± 0.906 pg/ml), (15. 41450± 0.218 pg/ml), (25. 4145065284± 0.218232 pg/ml) in compared to the control group (8.515 ± 0.515 ng/ml), (97.107565 ± 0. 700 pg/ml), (4.129558 ± 0. 190 pg/ml), (6.45839 ± 0. 220 pg/ml) as seen in table (1). Also the current study showed the serum concentration of NF-$_{KB}$ was correlated positively and significantly with serum concentration of IL-18, IL-1β and TNF-α. (r = 0.495), (r = 0.464) and (r = 0.486) as seen in Figure (1), (2) and (3).

Table 1
Concentration of human nuclear factor NF-kappa-B p65, IL-18, IL-1β and TNF-α Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group.

biomarkers	control	patient	p-value
NF-$_{KB}$ ng/ml	8.515±0.515	18.812±0.185	0.001
IL-18 pg/ml	97.107±0.700	474.123±0.906	0.001
IL-1β pg/ml	4.295±0.190	15.414±0.218	0.001
TNF-α pg/ml	6.458±0.220	25.652±0.232	0.001

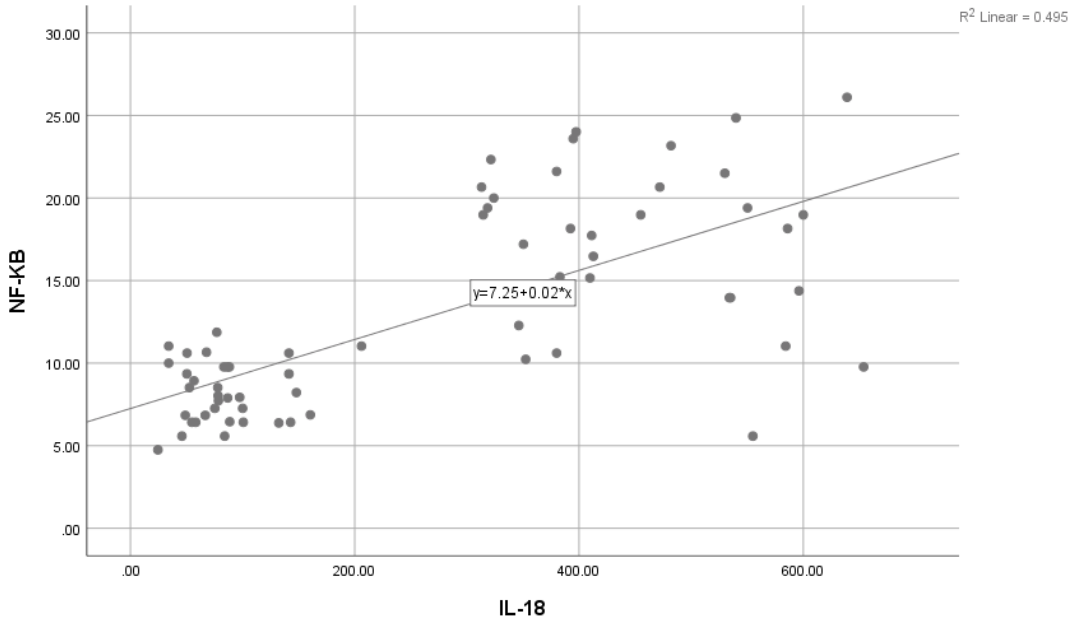

Figure 1. The Correlation between Serum Concentrations of NF-$_{KB}$ and Level of IL-18 in patients suffering from *Toxoplasma gondii* infection

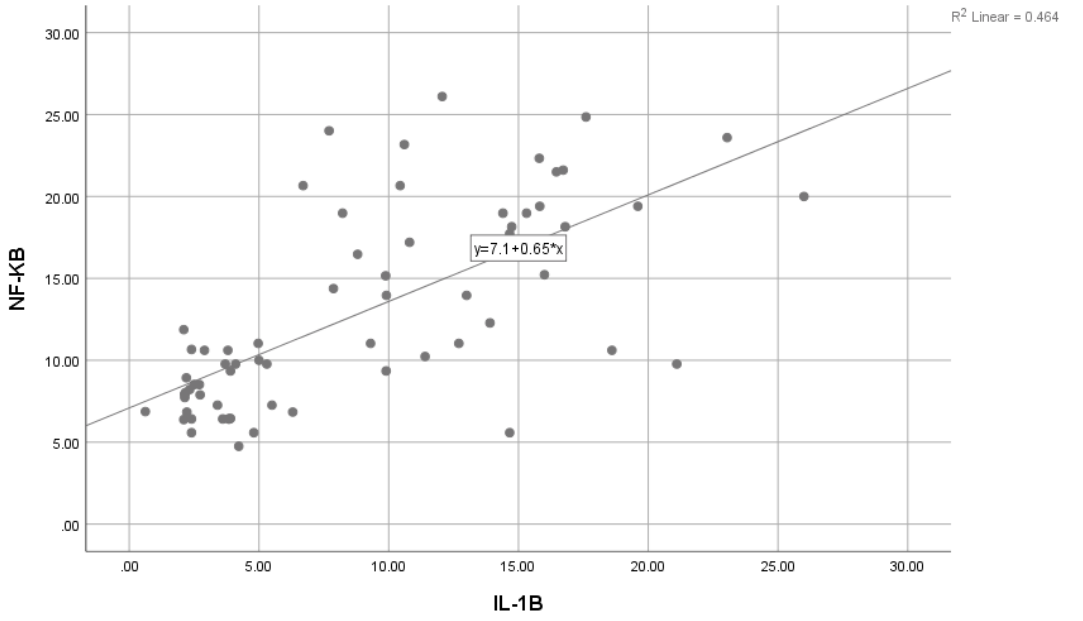

Figure 2. The Correlation between Serum Concentrations of NF-$_{KB}$ and Level of IL-1β in patients suffering from *Toxoplasma gondii* infection

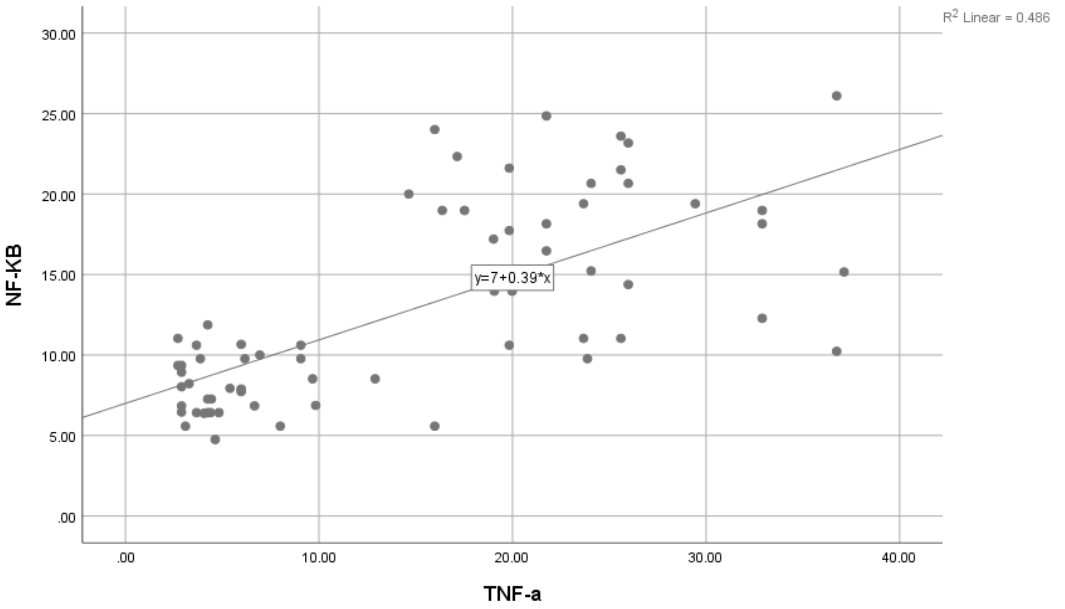

Figure 3. The Correlation between Serum Concentrations of NF-$_{KB}$ and Level of TNF-α in patients suffering from *Toxoplasma gondii* infection

Discussion

The current study showed the serum concentration of NF-$_{KB}$ was correlated positively and significantly with serum concentration of IL-18, IL-1β and TNF-α. (r = 0.495), (r = 0.464) and (r = 0.486)**,** Serum concentration of NF-$_{KB}$ correlated positively and significantly with IL-18 levels in the serum of patients suffering from *T. gondii* infection (r = 0.495). Pathogen associated molecular patterns (PAMPs) from Toxoplasma, such as profilin, are recognized by antigen presenting cells, leading to activation of downstream signaling pathways such as NF-κB. This in turn causes IL-12 secretion and activates natural killer cells and T cells to produce IFN-γ. IFN-γ is required for activation of downstream cell autonomous IFN-γ dependent toxoplasmacidal mechanisms and is essential for control of acute Toxoplasma infection. IFN-γ production by CD4+ and CD8+ T cells, part of adaptive immunity, is also essential for control of chronic Toxoplasma infection. Modified from (Yarovinsky, 2014). Sher *et al.* (1993) reported a Pathogen-activated antigen-presenting cells, particularly macrophages and DCs will induce the proliferation and stimulation of natural killer (NK) cells through IL-12 production in conjunction with TNF-α (Sher *et al.*, 1993), and this is enhanced by IL-18 production (Cai *et al.*, 2000). This will trigger a typical T helper type 1 (Th1) effector response, with IFN-γ-producing CD4 + T cells, and cytotoxic CD8 + T cells. IFN-γ is clearly the main mediator for resistance to *T. gondii* (Suzuki *et al.*, 1988).

The serum concentration of NF-$_{KB}$ correlated positively and significantly with IL-1β levels in serum of patients suffering from *T. gondii* infection. *T. gondii* can modulate the NF-κB pathway in different ways depending on the parasite strain. The dense granule protein GRA15 from type II *T. gondii* is an inducer of sustained

NF-κB activation (Rosowski *et al.*, 2011). Gov *et al.*, 2017 demonstrated, a role for the type II parasite-secreted GRA15 protein in NF-κB activation and IL-1β transcription in monocytes ,the differential IL-1β response of monocytes and neutrophils to *T. gondii* infection is intriguing, Although the precise mechanism by which *T. gondii* activates the inflammasome in human monocytes remains unknown, we have found that it depends on NLRP3, ASC, caspase-1, and K^+ efflux and appears to be "classical" inflammasome activation (Gov *et al.*, 2013; Gov *et al.*, 2017). Rosowski *et al.*, 2011 and Jensen *et al.*, 2011 demonstrate that NFκB activation leads to the transcription of pro-inflammatory genes, such as those encoding IL-1β and IL-12 (Rosowski *et al.*, 2011; Jensen *et al.*, 2011). In contrast, Butcher *et al.*, 2001 and Shapira *et al.*, 2002 demonstrate that the Type I *T. gondii* impairs the ability of LPS to activate NF-κB and to induce IL-12 and TNF-α in macrophages (Butcher *et al.*, 2001 ; Shapira *et al.*, 2002).

Serum concentration of $NF\text{-}_{KB}$ correlated positively and significantly with TNF-α levels in the serum of patients suffering from *T. gondii* infection (r = 0.486). It had been reported that TNF-*a* induces NF-*κβ* by one of two pathways, the canonical (classical) or the noncanonical. The former pathway is induced early after infection where NF-*κβ* dissociates from its inhibitory protein (I*κβ*) and translocates to the nucleus (Beg *et al.*, 1993; Wang *et al.*, 1998; Higashi *et al.*, 2010). The noncanonical NF-*κβ* pathway involves lymphopoiesis and differentiation of immune cells (Higashi *et al.*, 2010; Umran *et al.*, 2016). That seems to be in accordance with the recruits of T cells speculated precisely around the microvasculatures of the amygdala in the present work. Accordingly, chronic toxoplasmosis seems to be vitally regulated by the TNF-*a*/NF-*κβ* pathway.

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